Front. Chem. China (2006) 2: 193–198
DOI 10.1007/s 1458-006-0008-y

RESEARCH ARTICLE

Yu Wanying, Zhang Hua, Huang Weidong, Chen Jiping, Liang Xinmiao

Analysis of the volatile oil from the stem of *acanthopanax senticosus* (Rupr. et Maxim.) harms with several hyphenated methods of chromatography

Abstract Gas chromatography/quadrupole mass spectrometry (GC/qMS), gas chromatography/Fourier transform infrared spectrometry (GC/FTIR) and gas chromatography/orthogonal acceleration time-of-flight mass spectrometry (GC/oaTOFMS) were applied to analyze the volatile oil from the stem of *Acanthopanax Senticosus* (Rupr. et Maxim.) Harms. Based on the mass spectra search function of GC/qMS with the aid of the discriminability of the geometrical isomer by GC/FTIR and the ability to determine the accurate mass charge ratio *(m/z)* by GC/oaTOFMS, 68 GC eluants were identified successfully. Compared with the results obtained by GC/qMS only, the analytical results obtained by these hyphenated methods of gas chromatography are more credible.

Keywords gas chromatography/quadrupole mass spectrometry, gas chromatography/ fourier transform infrared spectrometry, gas chromatography/orthogonal acceleration time-of-flight mass spectrometry, *Acanthopanax senticosus* (Rupr. et Maxim.) harms, volatile oil

1 Introduction

Gas chromatography/quadrupole mass spectrometry (GC/qMS) has been used widely in the analysis of volatile oil that is obtained from traditional Chinese medicine (CTM). However, the qualitative results based on the spectra search function of GC/qMS usually are not entirely reliable, especially because a large number of compounds with similar chemical structures exist in the volatile oil of CTM. Many similar and uncertain results of the unknown compounds by GC/qMS identification will be produced when the spectra search function is carried out. Gas chromatography/Fourier transform infrared spectrometry (GC/FTIR) is one of powerful tools in the analysis of the complex volatile compounds [1], and has been used earlier to analyze the volatile oil obtained from CTM [2–4]. It is significant in the discrimination of the geometrical isomers; in spite of the sensitivity of GC/FTIR with the light pipe interface, the former still cannot be compared with GC/qMS. Gas chromatography/orthogonal Acceleration time-of-flight mass spectrometry (GC/oaTOFMS) is a new hyphenated technique of GC that has been developed in recent years [5–7]. It can provide mass resolution with 7 000 (FWHM) higher than the resolution with 1000 (FWHM) of traditional GC/qMS. The average deviation of the mass measurement for small fragment ions is less than 0.005mDa, and the element composition of the GC eluant will be suggested. So it offers a new tool for the analysis of the complex volatile oil obtained from CTM. This paper presents the identification of volatile oil from *Acanthopanax Senticosus* (Rupr. Et Maxim.) Harms by GC-qMS, GC/FTIR and GC/oaTOFMS.

Translated from *Chinese Journal of Chromatography*, 2005, 23(2)(in Chinese)

Yu Wanying(✉)
Advanced Analytical Center, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023,China，
E-mail: yuwy@dicp.ac.cn

Chen Jiping(✉), Zhang Hua, Huang Weidong, Liang Xinmiao
Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023,China，
E-mail: chenjp@dicp.ac.cn

2 Experimental

2.1 Instruments and conditions

GC/qMS analysis was performed using a gas chromatography/quadrupole mass spectrometer (GC/qMS) (Trace MS 2000, Finnigan USA). The GC conditions were as follows: a 60m × 0.25mm i.d. × 0.25μm DB-5 fused silica capillary column (J&W, Scientific CA.USA); injector kept at 260℃; split ratio: 50:1; Helium was used as the carrier gas with a constant flow rate of 1.0mL/min; the oven

temperature was programmed at a rate of 2℃/min from 50℃ (held for 5 min) to 250℃. The MS conditions were as follows: the transfer line was kept at 280℃; the sample size was 1.0 μL; the electron impact (EI) ionization mode at 70 eV; the temperature of source was kept at 250℃; acquisition of data was carried out over a range of 13～429 Dalton (Da), the spectra search program was NIST98 mass data base.

GC/oaTOFMS analysis was performed using a gas chromatograph/orthogonal acceleration time-of-flight mass spectrometer (GCT, Micromass, UK). The chromatography conditions were as follows: a 60m × 0.25 mm i.d. ×0.25μm DB-5 fused silica capillary column (J&W Scientific CA, USA) and a split injector (split ratio: 100:1) kept at 260℃; Helium was used as the carrier gas with a constant flow rate of 1.3mL/min; the oven temperature was programmed at a rate of 3℃/min from 50℃ (held for 3 min) to 250℃ (held for 20min); the sample size was 0.2 μL. The mass spectrometer conditions were as follows: EI mode at 30eV; the temperature of the source was kept at 250℃; the transfer line was kept at 260℃; acquisition of date was carried out over a mass range of 50-400Da. Exact mass was determined using a lock mass at *m/z* 263.9871 obtained by continued infusion of perfluorotri-N- butylamine during GC program.

GC/FTIR analysis was performed using a gas chromatography/Fourier transform infrared spectrometry system (Spectrum GX, Perkin Elmer, USA) with a gas chromatography (Auto system XL, Perkin Elmer, USA) equipped with a 50m × 0.54mm i.d. SE-30 fused silica capillary column (DICP, CAS, China) and a split injector kept at 250℃; Helium was used as the carrier gas with a constant pressure of 53.7kPa; the oven temperature was programmed at a rate of 3℃/min from 50℃(held for 10 min) to 210℃(held for 30min); the temperature of the light pipe interface was kept at 230℃; the pressure of tail purge gas was kept at 136kPa; the sample size was 1.0 μL; the system was operated using a MCT detector (work temperature: liquid N_2); the acquisition of data was carried out over a scan range of 4000cm^{-1}～700 cm^{-1} at a resolution of 8.0 cm^{-1}.

2.2 Sample preparation

The root of *Acanthopanax senticosus* (Rupr. Et Maxim.) Harms used in this experiment came from the Qinling Mountain, Shanxi Province of China. The extraction of volatile oil was carried out according to the pharmacopoeia of the People's Republic of China, Part 1 (Appendix XD). The yield of the volatile oil is 0.05%.

3 Results and discussion

The total ion current chromatogram (TIC) of the volatile oil of *Acanthopanax senticosus* (Rupr. Et Maxim.) Harms by GC/qMS analysis is shown in Fig.1. Ninety-four GC peaks were found on the TIC. Fifty GC peaks were found by GC/FTIR analysis, and the Gram-Schmidt reconstructed chromatogram of GC/FTIR is shown in Fig. 2. One hundred and fifty GC peaks were found by the GC/oaTOFMS analysis, and the TIC obtained by GC-oaTOFMS is shown in Fig. 3. Among the 94 GC peaks obtained by GC-qMS, 68 GC peaks were identified qualitatively with the aid of analysis of GC/FTIR and GC/oaTOFMS. The results are listed in Table 1. Among the 68 GC peaks, the contents of 20 components are more than 1% in the volatile oil that was analyzed by GC.

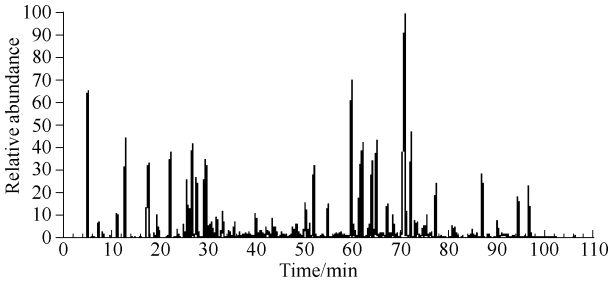

Fig.1 The total ion current chromatogram obtained by GC/qMS

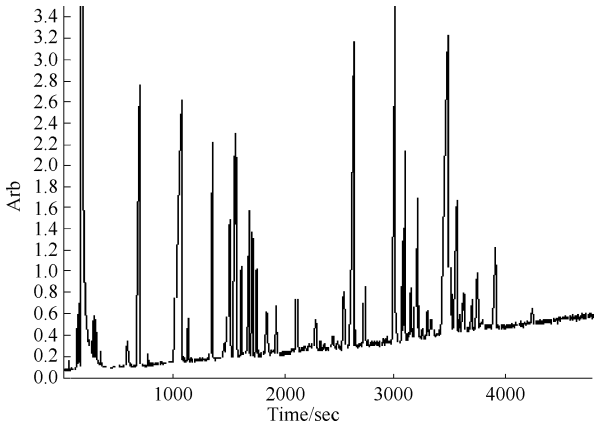

Fig. 2 The Gram-Schmidt reconstructed chromatogram obtained by GC/FTIR

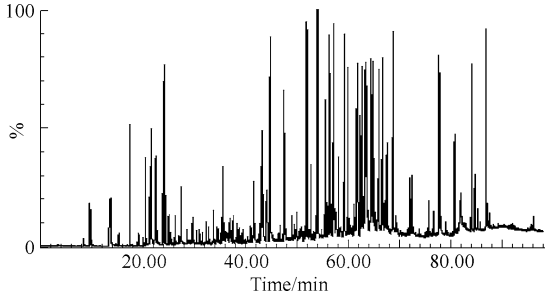

Fig. 3 The total ion current chromatogram obtained by GC/oaTOFMS

Table 1 The analytical results for the volatile oil from the stem of *Acanthopanax senticosus* (Rupr. et Maxim.) Harms by GC/qMS、GC/TOFMS and GC/FTIR

NO.	t_R /min[1)]	Compound	M_r	Formula	Area/%	Characteristic[2)] m/z(Da)	Proposed element composition	Deviation (mDa)	Method[3)]
1	7.24	Isovaleraldehyde	86	$C_5H_{10}O$	0.04	86.0735	$C_5H_{10}O$	0.3	1,2,3
2	7.46	Butyl alcohol	74	$C_4H_{10}O$	0.03	74.0761	$C_4H_{10}O$	2.9	1,2,3
3	8.38	Valeraldehyde	86	$C_5H_{10}O$	0.17	86.0406	$C_5H_{10}O$	3.3	1,2,3
4	9.80	Isopentyl alcohol	88	$C_5H_{12}O$	0.10	70.0733	$C_5H_{10}O$	5.0	1,2
5	9.96	2-Methyl-1-butanol	88	$C_5H_{12}O$	0.07	70.0750	$C_5H_{10}O$	3.3	1,2
6	11.32	1-Pentanol	88	$C_5H_{12}O$	0.05	70.0733	$C_5H_{10}O$	5.0	1,2,3
7	13.09	*n*-Hexanal	100	$C_6H_{12}O$	0.05	86.0726	$C_6H_{10}O$	5.7	1,2,3
8	14.96	Furfural	96	$C_5H_4O_2$	0.17	96.0263	$C_5H_4O_2$	5.2	1,2,3
9	17.86	*n*-Hexanol	102	$C_6H_{14}O$	0.54	84.0914	C_6H_{12}	2.5	1,2,3
10	18.89	2-Heptanone	114	$C_7H_{14}O$	0.15	114.0953	$C_7H_{14}O$	9.2	1,2,3
11	19.02	2-Butylfuran	124	$C_8H_{12}O$	0.18	124.0910	$C_8H_{12}O$	2.2	1,2
12	19.66	*n*-Heptaldehyde	114	$C_7H_{14}O$	2.63	114.1046	$C_7H_{14}O$	0.1	1,2,3
13	21.70	α-Thujene	136	$C_{10}H_{16}$	0.09	136.1318	$C_{10}H_{16}$	6.6	1,2
14	22.37	α-Pinene	136	$C_{10}H_{16}$	7.13	136.1252	$C_{10}H_{16}$	2.1	1,2,3
15	23.83	(Z)-2-Heptenal	112	$C_7H_{12}O$	0.06	112.0872	$C_7H_{12}O$	1.6	1,2
16	25.13	Heptanol	116	$C_7H_{16}O$	0.38	98.1107	C_7H_{14}	1.1	1,2,3
17	25.70	β-Pinene	136	$C_{10}H_{16}$	1.15	136.1231	$C_{10}H_{16}$	2.1	1,2,3
18	25.92	1-Octen-3-ol	128	$C_8H_{16}O$	0.23	110.1092	C_7H_{14}	1.4	1,2,3
19	26.45	6-Methyl-5-heptene-2-one	126	$C_8H_{14}O$	0.91	126.1032	$C_8H_{14}O$	1.3	1,2,3
20	26.90	2-*n*-Pentylfuran	138	$C_9H_{14}O$	3.09	138.1036	$C_9H_{14}O$	0.9	1,2,3
21	27.70	*n*-Caprylaldehyde	128	$C_8H_{16}O$	1.38	110.1081	C_8H_{14}	1.5	1,2,3
22	27.92	α-Phellandrene	136	$C_{10}H_{16}$	0.13	136.1257	$C_{10}H_{16}$	0.5	1,2
23	28.22	2,4-Heptadienal	110	$C_7H_{10}O$	0.05	110.0734	$C_7H_{10}O$	0.2	1,2
24	29.58	*p*-Cymene	134	$C_{10}H_{14}$	3.52	134.1082	$C_{10}H_{14}$	1.4	1,2,3
25	29.93	Limonene	136	$C_{10}H_{16}$	1.01	136.1245	$C_{10}H_{16}$	0.7	1,2,3
26	30.40	(cis)- β-Ocimene	136	$C_{10}H_{16}$	0.12	136.1287	$C_{10}H_{16}$	3.5	1,2
27	30.91	Benzeneacetaldehyde	120	C_8H_8O	0.45	120.0548	C_8H_8O	2.7	1,2,3
28	31.64	Melonal	140	$C_9H_{16}O$	0.08	140.1249	$C_9H_{16}O$	4.8	1,2
29	32.02	(E)-2-Octenal	126	$C_8H_{14}O$	0.41	126.1036	$C_8H_{14}O$	0.9	1,2,3
30	32.92	*p*-Tolualdehyde	120	C_8H_8O	0.05	120.0563	C_8H_8O	1.2	1,2
31	35.48	Linalool	154	$C_{10}H_{18}O$	2.09	136.1231	C_9H_{16}	2.1	1,2,3
32	35.71	*n*-Nonaldeyde	142	$C_9H_{18}O$	0.02	124.1285	C_9H_{16}	3.3	1,2
33	36.69	1-methyl-3-cyclohexene-1-carbox aldehyde	124	$C_8H_{12}O$	0.19	124.0877	$C_8H_{12}O$	1.1	1,2,3
34	37.88	4-Acetyl-1-methyl-cyclohexene	138	$C_9H_{14}O$	0.05	138.1044	$C_9H_{14}O$	0.1	1,2
35	38.72	Pinocarveol	152	$C_{10}H_{16}O$	0.07	152.1230	$C_{10}H_{16}O$	2.9	1,2
36	40.10	(E)-2-Nonenal	140	$C_9H_{16}O$	0.25	140.1229	$C_9H_{16}O$	2.8	1,2,3
37	40.51	Pinocarvone	150	$C_{10}H_{14}O$	0.03	150.0938	$C_{10}H_{14}O$	10.7	1,2
38	41.70	*p*-Menth-1-en-4-ol	154	$C_{10}H_{18}O$	0.05	154.1418	$C_{10}H_{18}O$	6.0	1,2
39	43.62	*n*-Decaldehyde	156	$C_{10}H_{20}O$	0.30	156.1571	$C_{10}H_{20}O$	5.7	1,2
40	44.27	(E,E)-Nonadienal	138	$C_9H_{14}O$	0.12	138.1028	$C_9H_{14}O$	1.7	1,2,3
41	45.46	6,7-Dimethyl-1,2,3,5,8,8a-hexahydronaphthalene,	162	$C_{12}H_{18}$	0.04	162.1392	$C_{12}H_{18}$	1.7	1,2
42	49.11	Phellandral	152	$C_{10}H_{16}O$	0.03	152.1217	$C_{10}H_{16}O$	1.6	1,2
43	49.79	Anisole	148	$C_{10}H_{12}O$	0.01	148.0871	$C_{10}H_{12}O$	1.7	1,2

Table 1 (Continued)

NO.	t_R /min[1)]	Compound	M_r	Formula	Area/%	Characteristic[2)] m/z(Da)	Proposed element composition	Deviation (mDa)	Method[3)]
44	50.32	(Z,Z)-2,4 Decadienal	152	$C_{10}H_{16}O$	1.51	152.1196	$C_{10}H_{16}O$	0.5	1,2,3
45	51.25	Carvacrol	150	$C_{10}H_{14}O$	0.41	150.1045	$C_{10}H_{14}O$	0.0	1,2
46	52.21	(E,E)-2.4-Decadienal	152	$C_{10}H_{16}O$	7.90	152.1219	$C_{10}H_{16}O$	1.8	1,2,3
47	55.06	Eugenol	164	$C_{10}H_{12}O$	0.98	164.0805	$C_{10}H_{12}O$	3.2	1,2
48	55.21	dihydro-5-pentyl-2(3H)-Furanone	156	$C_9H_{16}O_2$	0.11	85.0251	$C_4H_5O_2$	3.9	1,2
49	59.95	Isocaryophyllene	204	$C_{15}H_{24}$	9.97	204.1896	$C_{15}H_{24}$	1.8	1,2,3
50	60.44	2.6-dimetyl-6-(4-methyl-3-pentenyl) 2-Norpinene	204	$C_{15}H_{24}$	0.25	204.1860	$C_{15}H_{24}$	1.8	1,2
51	61.26	Geranylacetone	194	$C_{13}H_{22}O$	0.07	194.1686	$C_{13}H_{22}O$	1.5	1,2
52	61.66	β-Farnesene	204	$C_{15}H_{24}$	5.09	204.1887	$C_{15}H_{24}$	0.9	1,2,3
53	62.17	Humulene	204	$C_{15}H_{24}$	4.50	204.1860	$C_{15}H_{24}$	1.8	1,2,3
54	63.46	2-Heptene,2-methyl-p-tolyl	202	$C_{15}H_{22}$	0.04	202.1687	$C_{15}H_{22}$	3.5	1,2
55	64.12	3,7,11-Trimethyl-1,3,6,10-dodecatet raene	204	$C_{15}H_{24}$	1.11	204.1880	$C_{15}H_{24}$	1.0	1,2,3
56	65.24	β-Bilopanone	204	$C_{15}H_{24}$	0.08	204.1870	$C_{15}H_{24}$	0.8	1,2
57	67.32	β-Caryophyllene	204	$C_{15}H_{24}$	0.16	204.1830	$C_{15}H_{24}$	4.8	1,2
58	70.95	Caryophyllene oxide	220	$C_{15}H_{24}O$	16.4	220.1822	$C_{15}H_{24}O$	2.5	1,2,3
59	72.36	Humulene oxide	220	$C_{15}H_{24}O$	4.83	138.1020	$C_9H_{14}O$	2.5	1,2,3
60	73.08	Spathulenol	220	$C_{15}H_{24}O$	0.09	220.1813	$C_{15}H_{24}O$	1.4	1,2
61	77.41	Tetradecanal	212	$C_{14}H_{28}O$	3.74	182.2012	$C_{13}H_{26}$	2.3	1,2,3
62	80.87	*n*-pentadecanol	228	$C_{15}H_{32}O$	0.35	210.2327	$C_{15}H_{30}$	2.1	1,2,3
63	81.44	10,12-Hexadecadienal	236	$C_{16}H_{28}O$	0.32	236.2189	$C_{16}H_{28}O$	4.9	1,2
64	84.93	11,13-Hexadecadien-1-ol	238	$C_{16}H_{30}O$	0.15	238.2335	$C_{16}H_{30}O$	3.8	1,2
65	87.01	9,17-Octadecadienal	264	$C_{18}H_{32}O$	2.79	250.2313	$C_{17}H_{30}O$	1.6	1,2
66	90.21	9,12-Octadecadien-1-ol	266	$C_{18}H_{34}O$	0.32	234.2373	$C_{17}H_{30}$	2.5	1,2
67	94.40	Manoyl oxide	290	$C_{20}H_{34}O$	2.50	290.2594	$C_{20}H_{34}O$	1.6	1,2
68	96.70	*n*-Octadecanol	270	$C_{18}H_{38}O$	2.66	252.2810	$C_{18}H_{36}$	0.7	1,2

1) The retention time obtained by GC/qMS. 2) The results obtained by GC/oaTOFMS. 3) Methods: 1.GC/qMS; 2. GC/oaTOFMS; 3.GC/FTIR.

Due to the limitation of sensitivity, as a secondary method in this paper, GC/FTIR was used to check and confirm the results from GC-qMS identification, especially when several similar results that cannot be discriminated by GC-qMS emerged. For example, there were three similar answers: 2-allyl-6-methoxyphenol(A),4-allyl-2- methoxyphenol (B) and 2-methoxy-4- propenylphenol (C)) for the GC peak at 55.06 min obtained by GC-qMS identification (Fig.1) using the NIST98 spectra search program. It is very difficult to ascertain which is the right answer. However, it is easy to identify that the compound of the GC peak at 55.06 min is 4-allyl -2-methoxyphenol (B) by comparing the spectra from the GC/FTIR with the standard vapor phase infrared spectra.

The results of mass spectra search of GC/qMS for the eluants at 50.32 min and 52.21 min (GC peaks 44# and 46#) gave the entire same mass spectra and showed that both are E,E 2,4-nonenal with higher level of matching (Fig. 5). But it is impossible that one compound has two different retention times under the same GC condition. So it is suggested that these two eluants may be the same compound

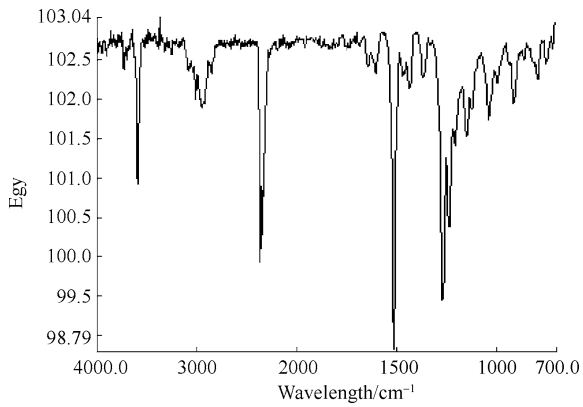

Fig. 4 The IR spectrum obtained by GC/FTIR corresponding to the GC peak with retention time of 55.06 min in Fig. 1

of different geometric isomers. After the normalization analysis of the infrared spectra (Fig. 6) corresponding to the GC peak 44# and 46# in the TIC obtained by GC/qMS, the above suggestion is confirmed. Observing, in detail, the two characteristic absorption peaks in the range of 1600 cm^{-1} to

1650 cm^{-1} , it is clear that there is a difference between the two infrared spectra—in comparing with GC peak 46#, the two characteristic absorption peaks of GC peak 44# move to a lower wave number. So it is confirmed that GC peak 44# is mainly Z,Z 2,4-nonenal and GC peak 46# is mainly E,E 2,4-nonenal.

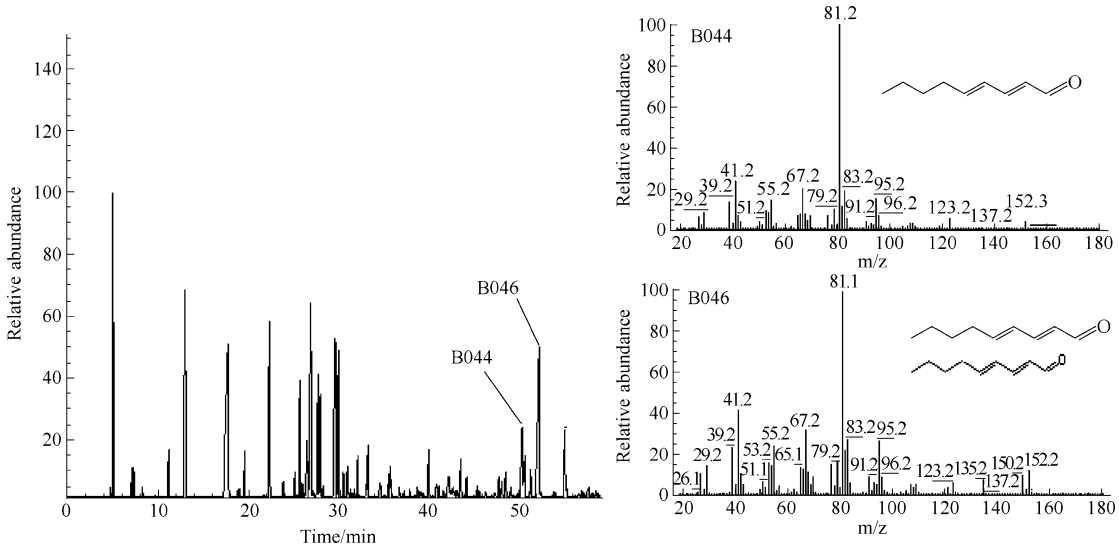

Fig. 5 Analytical results of compounds 44# and 46# in Table 1 by GC/qMS

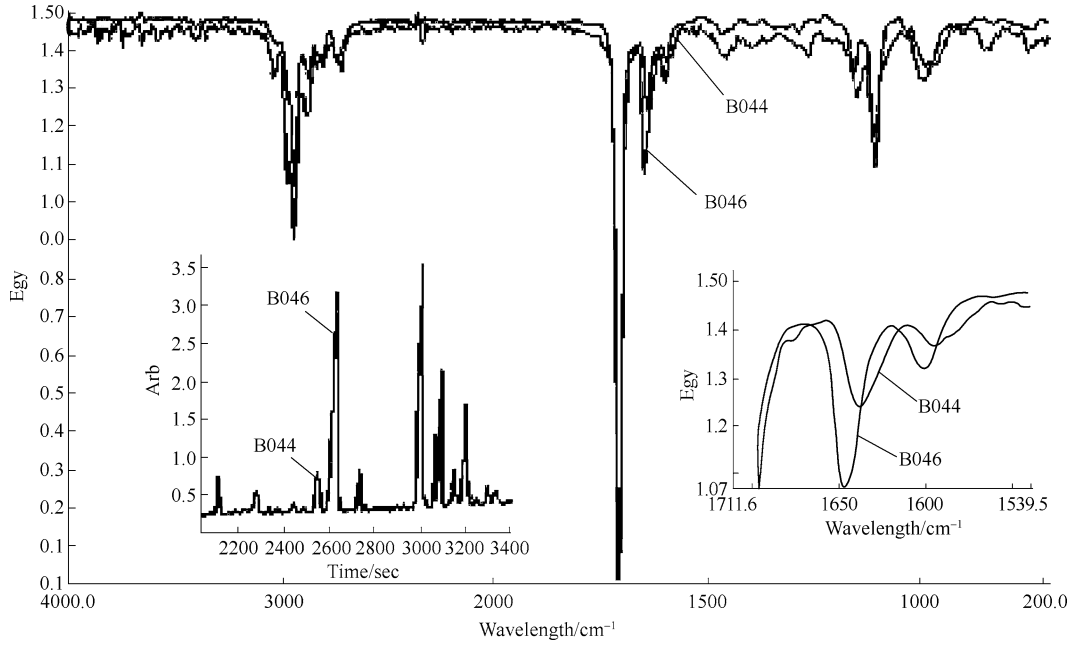

Fig. 6 The results of compounds 44# and 46# in Table 1 by GC/FTIR

There are about 10,000 standard vapor phase infrared spectra, but only a few spectra can be applied to the analysis of CTM. However, the GC/FTIR analysis can also be used to obtain some information about the chemical structure of the GC eluants. In this experiment the GC eluants identified by GC/qMS were validated by GC/FTIR again. For example, the chemgram (selective functional chromatogram) between the range of 1660 cm^{-1} and 1800 cm^{-1} (Fig. 7) obtained by GC/FTIR shows that there are more than 40 GC components, which consist of the C = O functional group, in the volatile oil. Especially, some eluants that sometimes cannot be observed in the whole spectral range (4000 cm^{-1} ~ 700 cm^{-1}) may be detected on the chemgram, and some overlapping component existing in one GC eluant may likely be discriminated using this selective functional chromatogram.

GC/oaTOFMS is a new method to measure the exact mass of small fragment ions. The deviation of GC-oaTOFMS analysis is usually below 5 mDa, so the elemental compositions of molecules and fragment ions can

be prompted. GC-oaTOFMS analysis can also be used to unveil the principle of dissociation and the interpretation of the mass spectra. In this experiment the exact mass measurement for the molecular ion of each GC peak was carried out by GC-oaTOFMS, then the elemental composition of each molecule or main fragment ion was obtained, and the average deviation was 2.55mDa. At last, the results analyzed by GC/qMS and GC/FTIR were validated using the data of elemental composition by GC-oaTOFMS to obtain the most reliable analysis results.

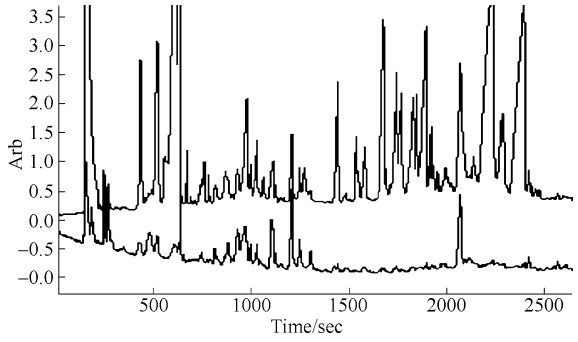

Fig. 7 The Gram-Schmidt reconstructed chromatogram (upper) and the selective functional chromatogram (1 660-1800cm^{-1}) (lower) obtained by GC/FTIR

4 Conclusions

The volatile oil in the root of *Acanthopanax senticosus* (Rupr. Et Maxim.) Harms from the Shanxi Province of China was analyzed by three different kinds of hyphenated GC techniques. Sixty-eight GC eluants were identified successfully. Since the analytical results of any one qualitative method are not infallible, it is usually not entirely reliable to use a single analytical technique. In spite of the sensitivity of GC/FTIR with the light pipe interface,GC/FTIR cannot be compared with GC-qMS; as a validation and supplementary tool, it is still useful in the identification of GC eluants. With the capability of precise measurement on *m/z* value of GC-oaTOFMS, the results identified by GC-qMS and GC/FTIR were further validated. This paper shows that for a complex mixture system such as the volatile oil of CTM, the results of qualitative analysis will be more reliable by using a multi-analytical technique.

References

1. Wu Jinguang, Technology of Modern FTIR and Its Application, Beijing, Scientific and Technical Publishing House，1994, 187−189
2. Wu Qiao, Qiu Ningying，Ge Zhaoheng, Acta Pharmaceutica Sinica, 1990, 25(1): 44−48
3. Zhong Shan，Feng Zigang, Chinese Journal of Analytical Chemistry, 1995, 23(2): 132−136
4. Liu Mixin，Wang Wei, Spectroscopy and Spectral Analysis, 1996, 16(5): 35–37
5. Fay L. B., Newton A., Simian H., Robert F., Douce D., Hancock P., Green M., Blank I., J. Agric. Food Chem., 2003, 51(9): 2708−2713
6. Gu Mingsong, Zhang Li, Hu Xuying, Feng Jianlin, Liu Yintang, Xie Jianwei, Journal of Chineses Mass Spectrometry Society, 2004, 25(1): 48−51
7. Yu Wanying, Zhang Hua, Huang Weidong, Chen Jiping, Liang Xinmiao. Talanta, 2005, 65: 172−178
8. Pharmacopoeia of the People' s Republic of China Part 1(Appendix XD). Beijing: Chemical Industry Press，2000
9. Sadtler Standard Infrared Vapor Phase Spectra, No: 3 539; No: 2 906; No: 134